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# FEASIBILITY STUDY TO ESTIMATE PERSON-TO-PERSON STABILITY OF mRNA SIGNATURES OF RADIATION EXPOSURE IN HUMANS

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# LDRDProjectFinalReport

## FEASIBILITYSTUDYTOESTIMATEPERSON -TO-PERSON STABILITYOFmRNASIGNATURESOFRADIATIONEXPOSURE INHUMANS

TrackingCodenumbe03 -FS-029

AccountNumber:3939 -55

### Purpose:

Thepurposeoftheresearchistoconduct twostudiesatareimportantforestablishingthe feasibilityofgeneexpressionprofilesasbiodosimetersofexposuretoionizingradiation.

**Aim1** . Tomeasureperson -to-personvariabilityingenetranscriptresponsetoradiation.

**Aim2** . Measurethe effectsovertimeafterradiationongeneexpressionprofiles.

### Background:

Geneexpressionisknowntochangewithtimeanddoseafterexposuresuggestingthatitcan beusedtoassessexposeddoseinindividualsafteranexposureevent.However,therei s insufficientinformationtoassesstheapplicabilityofthisapproachtothegeneralpopulation. Understandingtheeffectsovertimeafterradiationandgene -expressionvariabilityamong individualsisimportantinassessingthefeasibilityofmRNAexpress ionprofilesasbiological dosimetersofexposuretoradiation.Weproposedtoinvestigategeneexpressionvariationin mRNAsignaturesafterexposuretoionizingradiationusingawell characterizednational collectionofhumanlymphoblastoidcelllineso btainedfromadiversepopulationofadults.The overallgoalistounderstandthebiologicalvariationaswellastemporalaspectsspecifictothe adaptiveresponseprocessovertimeof48hours.

HumanLymphoblastoid(HLB)cellswereobtainedfrom theNIHHumanGeneticCell Repository. TheHumanGeneticCellRepositoryresourceiscomprisedofcelllinesfrom450 unrelatedindividuals,maleandfemalestodesignedtoreflectthediversityinthehumanand facilitatesfindinggeneticvariantsintheent irehumanpopulationfromarandomsampleof residentsoftheUnitedStates.

Weutilizedgenetranscriptmicroarraysrepresentingapproximately22,000humangenesper arraytodevelopstandardcurvesandcharacterizetheeffectsofvariablesimportantfor the applicationofradiationdosimetry:exposeddose,timeafterexposure,andindividualvariation. Theprimaryfocusofthisstudywastoassessinter -individualvariationatseveralselected dosesandtimepointsafterexposuretohelpdevelopbiodosi meters.

### Activities:

#### ExperimentalDesignandwetlabwork:

Aims1and2Areintegratedintoasinglestudydesignthatimprovestheefficiencyand minimizestheDNAchipcosts.Sixcelllines:twoadaptive,threenon -adaptive,andonethat was"synergistic"asdeterminedinthestudyofSorensonetal.,2002.

-Threeexposures:0cGy,10cGyand200cGy.

-Post -exposuretimepoints:4hours,and24hoursforallcelllines.

-Eightpost -exposuretimepoints(15',30',1h,2h,4h,8h,24h,48h)foronead aptiveandone non-adaptivecelllinewereinvestigated.

# Progress Report:

Vials of human lymphoblastoid cells were thawed and grown in culture until they were expanded and irradiated using a cesium source. Sixty milliliters of cells in suspension culture, in a plastic tissue culture flask, were irradiated at specific doses following which they were incubated at 37 °C for defined times and then harvested. Cells were spun down, washed with Phosphate Buffered Saline, aliquoted into 6 tubes each and spun down. Dry pellets were flash frozen in liquid nitrogen, and transferred to -80 °C. RNA is isolated from frozen cell pellets using standard protocols, and quantified/checked for quality with the Agilent Bioanalyzer and Spectrophotometer.

**Samples Collected:** Biodosimetry Project: 2X3X8=48 samples; Interindividual Variation Project: 6X3X2=36 samples. Experimental control and test target messenger RNA were labeled using a T7 amplification kit (Arcturus Inc.). Amplification followed by biotin labeling was performed to generate targets. Targets were next purified and fragmented according to the Affymetrix protocol. Labeled and fragmented RNA was checked to ensure quality. Microarrays were hybridized, washed and scanned for signal following the Affymetrix protocol using Affymetrix human GeneChips (HGU133A). MAS 5.0 chip reports were generated and quality metrics performed to ensure quality of hybridizations.

**Statistical & Bioinformatic Analyses:** Data analysis proceeded in 3 stages.

Phase 1: *Quality Assurance*: First, the data was quality checked and normalized with the best available algorithms. Our current approach is to use RMA (robust multiarray averaging) from the affymetrix package on the BioConductor website.

Phase 2: Second, the data is currently being filtered using robust linear model techniques to obtain sets of genes exhibiting statistically meaningful expression differences across time points and cell lines.

Phase 3: *Exploratory and Discovery Techniques*: The resulting subset of the data will then be analyzed using a variety of exploratory and model-based methods to discover sets of genes with common patterns of expression and statistically meaningful interactions and correlations across time points. Inter-cell line variation will be assessed using random and mixed effect linear models. Genes that show robust response patterns across cell lines will become candidates for biodosimeters and validation by single-cell methods. Genes that show person-to-person differences will be evaluated as indicators of differential individual response.

Phase 1 of the Statistical Analysis is completed, Phase 2 and 3 are in process.